# Paragrillotia apecteta n. sp. and redescription of *P. spratti* (Campbell & Beveridge, 1993) n. comb. (Cestoda, Trypanorhyncha) from hexanchid and carcharhinid sharks off New Caledonia

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## **ABSTRACT**

A new species of *Paragrillotia* Dollfus, 1969, *P. apecteta* n. sp., is described from the spiral valve of *Hexanchus nakamurai* Teng, 1962, from Lifou, New Caledonia, South Pacific. The new species differs from congeners in its distinctive basal armature with characteristic enlarged hooks, the presence of seven hooks per principal row and the presence of two or three rows of intercalary hooks. *Pseudogrillotia spratti* Campbell & Beveridge, 1993 is redescribed from specimens collected from *Negaprion acutidens* (Rüppell, 1837) from New Caledonia. The fully mature segment is described for the first time. The presence of a chainette on the external surface of the tentacle indicates that the species belongs to *Paragrillotia* rather than to *Pseudogrillotia* Dollfus, 1969 as *Paragrillotia spratti* (Campbell & Beveridge, 1993) n. comb. Both species represent the first records of the genus *Paragrillotia* from the southern hemisphere.

KEY WORDS
Cestoda,
Trypanorhyncha,
Paragrillotia,
Paragrillotia apecteta n. sp.,
Paragrillotia spratti,
New Caledonia,

new species.

## RÉSUMÉ

Paragrillotia apecteta n. sp. et redescription de P. spratti (Campbell & Beveridge, 1993) n. comb. (Cestoda, Trypanorhyncha) de requins Hexanchidae et Carcharhinidae de Nouvelle-Calédonie.

Une nouvelle espèce de *Paragrillotia* Dollfus, 1969, *P. apecteta* n. sp., est décrite de la valvule spirale d'un *Hexanchus nakamurai* Teng, 1962, de Lifou, Nouvelle-Calédonie, Pacifique Sud. La nouvelle espèce diffère de ses congénères par son

MOTS CLÉS Cestoda, Trypanorhyncha, Paragrillotia, Paragrillotia apecteta n. sp., Paragrillotia spratti, Nouvelle-Calédonie, nouvelle espèce. armature basale distinctive avec des grands crochets caractéristiques, la présence de sept crochets par rangée principale et la présence de deux à trois rangées de crochets intercalaires. *Pseudogrillotia spratti* Campbell & Beveridge, 1993 est redécrit à partir de spécimens récoltés chez *Negaprion acutidens* (Rüppell, 1837) de Nouvelle-Calédonie. Le segment pleinement mûr est décrit pour la première fois. La présence d'une chaînette sur la surface externe du tentacule indique que l'espèce appartient à *Paragrillotia* plutôt qu'à *Pseudogrillotia* Dollfus, 1969 et devient *Paragrillotia spratti* (Campbell & Beveridge, 1993) n. comb. Les deux espèces représentent les premières mentions du genre *Paragrillotia* dans l'hémisphère sud.

## INTRODUCTION

Currently, the trypanorhynch genus *Paragrillotia* Dollfus, 1969 is monotypic, with the only known species *P. similis* (Linton, 1909) (syn. *Grillotia* (*Paragrillotia*) *simmondsi* Dollfus, 1969) occurring in the spiral valve of the nurse shark, *Ginglymostoma cirratum* (Bonnaterre, 1788), from off the coast of Florida (Linton 1909; Dollfus 1969; Caira & Gavarrino 1990).

Recent collections of cestodes from sharks off New Caledonia have revealed a new species of *Paragrillotia*, described herein. In addition, a redescription of *Pseudogrillotia spratti* Campbell & Beveridge, 1993, based on specimens collected off New Caledonia, suggests that it belongs to the genus *Paragrillotia* rather than to *Pseudogrillotia* Dollfus, 1969. Both species are described below and their allocation to the genus *Paragrillotia* is discussed.

## MATERIALS AND METHODS

A single specimen (total length 166 cm) of a six-gill shark *Hexanchus nakamurai* Teng, 1962 was collected during the CHONDRICAL cruise of the NO *Alis* organised by the Institut de Recherche pour le Développement (IRD) off New Caledonia in January and February 2002. The shark was examined on board immediately after capture. Its isopods were reported in Trilles & Justine (2004). The spiral valve was dissected and cestode specimens

were collected and fixed in 70% ethanol. A single specimen (total length 270 cm) of a lemon shark, *Negaprion acutidens* (Rüppell, 1837) was caught off Nouméa, New Caledonia (precise locality unknown). The spiral valve was fixed by injection of 4% formalin and kept in formalin until dissection and collection of cestodes.

Cestode specimens for preparation as whole mounts were stained in celestine blue, dehydrated in ethanol, cleared in methyl salicylate and mounted in Canada balsam. Serial sections were prepared from mature segments embedded in paraffin and were sectioned at a thickness of 5  $\mu$ m. Sections were stained with haematoxylin and eosin. Individual tentacles were removed with a scalpel blade and mounted in glycerine jelly.

Morphological terminology for trypanorhynch cestodes follows Dollfus (1942) and Campbell & Beveridge (1994) except that the attachment organs are referred to as bothria following Jones *et al.* (2004).

Drawings were made with the aid of a drawing tube attached to an Olympus BH microscope. In dorso-ventral views of the segments, only the vitelline follicles in the lateral regions of the segment are shown. Measurements were made with an ocular micrometer. All measurements are presented in µm, unless otherwise stated, as the range followed, in parentheses, by the mean and the number of specimens measured (n). Specimens have been deposited in the Muséum national d'Histoire naturelle, Paris (MNHN). Host nomenclature follows Eschmeyer

(1998). Types of *P. spratti* in the South Australian Museum, Adelaide (SAM) were re-examined.

## **SYSTEMATICS**

# *Paragrillotia apecteta* n. sp. (Figs 1; 2)

MATERIAL EXAMINED. — Holotype from spiral valve of *Hexanchus nakamurai*, Baie du Santal, Lifou, New Caledonia, 20°53'30"S, 167°01'60"E, depth 298-397 m, coll. J.-L. Justine, 26.I.2002 (MNHN JN 01F1). — Same data, 3 paratypes, on 5 slides (MNHN JN 01F2-JN 01F6).

ETYMOLOGY. — The species name, *apecteta*, is derived from the Greek *apectetos*, meaning unkempt or uncombed and alludes to the complex array of hooks on the external surface of the tentacle.

## DESCRIPTION

Based on 4 immature specimens with up to 11 segments. Small cestodes, up to 25 mm long, maximum width 550. Scolex acraspedote, 4.3-5.4 (4.9, n = 4) mm long; maximum width in pars bulbosa, 760-940 (810, n = 4); 2 oval bothria with thickened margins and median indentation in posterior margin; pars bothrialis 940-1020 (980, n = 4) long; pars vaginalis 2.10-2.95 (2.63, n = 4) mm long; tentacular sheaths straight in anterior part of pars vaginalis, coiled in posterior part; bulbs elongate, 1.6-2.0 (1.81, n = 4) mm long, width 260-340 (300, n = 4); bulb length: width ratio 1:5.2-7.4 (6.3, n = 4); prebulbar organ and glands within bulb absent; retractor muscle inserts in mid-region of bulb, continued posteriorly on internal surface of bulb by row of cells; pars post-bulbosa absent; mean scolex ratio (pars bothrialis:pars vaginalis: pars bulbosa) 1:2.7:1.8.

Tentacles with slight basal swelling; everted tentacles up to 1100 long, 170-240 (200, n = 5) in diameter at base, 140-170 (160, n = 5) in diameter in metabasal region. Tentacular armature heteroacanthous, heteromorphous; hooks hollow; hook rows appear to begin on internal surface, terminate on external surface of tentacle. Distinctive basal armature present; initial 2 rows of hooks slender, elongate on internal surface, diminishing in size

towards external surface; third row of hooks with characteristic, enlarged hooks of unusual shapes; principal rows commence at fourth row; on external surface, hook rows more numerous than on internal surface at base, hooks of external surface sagittate, 18-53 (39, n = 5) long, base 5-15 (9, n = 5) wide; hooks become smaller distally; at level of hook row 3 on external surface, hooks small, uncinate, arranged in compact array, approximately 12 rows long and 6 columns wide. Hook rows of metabasal region consist of 7 hooks; space present between hook files 1(1') on internal surface; hooks 1(1') large, robust, uncinate with elongate base, 105-125 (112, n = 5) long, base 80-90 (84, n = 5) long; hooks 2(2') similar in shape but smaller, 78-93 (82, n = 5) long, base 50-55 (53, n = 5) long; hooks 3(3') erect, broadly falcate, with narrower base 75-123 (102) long, base 20-33 (31, n = 5) long; hooks 4(4') slender, falcate with narrow base, 78-115 (96, n = 5) long, base 20-28 (24, n = 5) long; space frequently present between hooks 4(4') and 5(5'); hooks 5(5') slender, variable in length, 68-165 (114, n = 5) long, base 18-35 (26, n = 5) long; hooks 6(6') slender, shorter, 58-125 (82, n = 5) long, base 10-25 (17, n = 5) long; hooks 7(7') slender, much shorter than hooks 6(6'), 29-38 (33, n = 5) long. Two or 3 rows of intercalary hooks, with 4-6 hooks in first 2 rows and up to 4 hooks in third row, if present; intercalary hooks begin at level of hooks 4(4'), continue just beyond hooks 6(6'); intercalary hooks 35-88 (69, n = 5) long, base 10-28 (16, n = 5) wide. External surface of tentacle with chainette of tiny hooks present in middle of surface with one or more chainette elements per principal row of hooks; chainette hooks uncinate 10-15 (13, n = 5) long, base 3 (3, n = 5) wide.

Segments craspedote, largest mature segment 2.57 mm long, maximum width 520; genital pores lateral, alternate irregularly, in posterior half of segment margin, 1050 (41%) from posterior end. Hermaphroditic sac 180-250 (220, n = 2) long, width 60-88 (74, n = 2); testes numerous, c. 400, filling entire medulla, with c. 50 testes posterior to ovary; testes confluent anterior to uterus; arranged in single layer, 43-60 (48, n = 5) in diameter. Ovary not fully developed, bi-lobed in dorso-ventral view, well anterior to posterior margin of segment. Uterus



Fig. 1. — Paragrillotia apecteta n. sp. from Hexanchus nakamurai Teng, 1962: A, external surface of tentacle, metabasal region; B, antibothrial surface of tentacle, metabasal region of tentacle with hooks of chainette shown as solid hooks; C, bothrial surface, basal region of tentacle; D, basal region of tentacle, antibothrial surface. Scale bar: 100 µm.

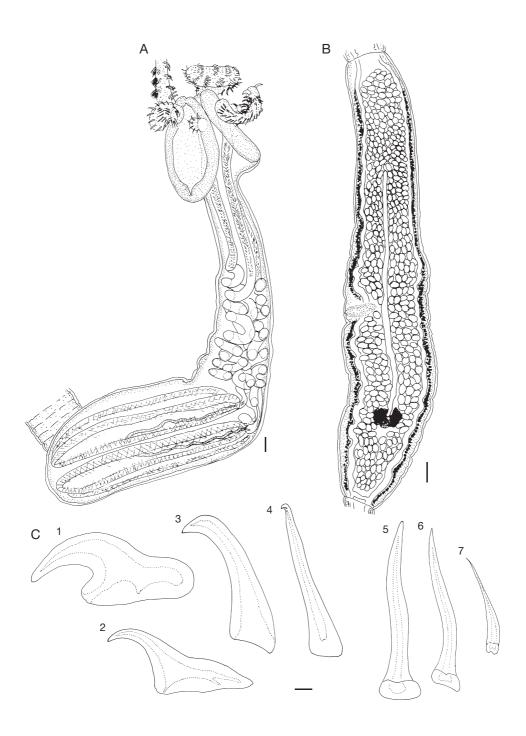


Fig. 2. — Paragrillotia apecteta n. sp. from Hexanchus nakamurai Teng, 1962: **A**, scolex; **B**, mature segment; **C**, hooks of principal rows. Scale bars: A, B, 100 µm; C, 10 µm.

median, tubular, extends from ovarian isthmus to anterior quarter of segment. Vitelline follicles circumcortical. Ventral osmoregulatory canal *c.* 30 in diameter; canals on each side of segment joined by transverse canal at posterior margin of segment.

## REMARKS

The species described herein is allocated to the genus *Paragrillotia* on the basis of possessing two bothria, a distinctive basal armature, rows of intercalary hooks and a chainette in the centre of the external surface of the tentacle. The species resembles the type species, *P. similis*, in having 6 or 7 hooks per principal row, with 6 in *P. similis* and 7 in *P. apecteta* n. sp. (compared with 10 in *P. spratti*) but differs in having more intercalary hooks, with 4-6 hooks in the first two rows and up to four hooks in third row, if present. By contrast, in *P. similis*, the first intercalary row consists of four hooks and the second row of one hook. In addition, the basal armature differs in lacking the enlarged hooks seen on the external surface of the tentacle of *P. similis*.

The description of the new species is limited by the immaturity of the specimens available and by their contracted nature. The tentacular armature appears to begin on the internal surface of the tentacle and terminate on the external surface, but this is difficult to determine and needs to be confirmed from additional collections. In addition, the terminal genitalia were not fully developed and it was not possible to determine whether a cirrus sac or an hermaphroditic sac was present. As congeners have an hermaphroditic sac, the same term has been applied in *P. apecteta* n. sp.

Paragrillotia spratti (Campbell & Beveridge, 1993) n. comb. (Figs 3; 4)

Pseudogrillotia spratti Campbell & Beveridge, 1993: 41-43, figs 10-16.

MATERIAL EXAMINED. — Paratypes from spiral valve of *Negaprion acutidens*, off Nouméa, New Caledonia, 15.V.2003, coll. J.-L. Justine, 18 specimens (SAM 23331) (balsam mounts on 17 slides, MNHN JNC 441E1-JNC 441E17; tentacles mounted in glycerine jelly on 5 slides

MNHN JNC 441E18-JNC 441E22; serial sections on 19 slides JNC 441E23-JNC 441E41).

## DESCRIPTION

Longest specimen 93 mm long, maximum width 1300, with 110 segments, terminal segments mature. Scolex either slightly craspedote or acraspedote, 4.45-6.00 (4.96, n = 10) mm long, maximum width in region of pars bulbosa 850-1100 (970, n = 10); 2 oval bothria with median indentation in posterior margin, pars bothrialis 800-950 (890, n = 10; pars vaginalis 2700-4270 (3240, n = 10), sheaths spiral; bulbs 1500-1820 (1680, n = 10) long, 250-320 (290, n = 10) wide; bulb length:width ratio 1:5.2-7.2 (1:5.9, n = 10); prebulbar organ absent; retractor muscle originates in anterior region of bulb, insertion continued posteriorly by band of tissue on internal surface of bulb; pars post bulbosa absent, bulbs frequently project into pars proliferans scolecis. Mean scolex ratio 1:3.60:1.89.

Tentacles incompletely everted, maximum length 700; tentacle diameter 130-180 (150, n = 10) at base; no basal swelling. Tentacular armature heteroacanthous, heteromorphous; hooks hollow; principal rows of 9 or 10 hooks beginning on internal surface of tentacle, terminating on external surface; space present between hook files 1(1') on internal surface. Hooks 1(1') large, uncinate, with broad base, 113-135 (128, n = 5) long, base 83-98 (93, n = 5) long; hooks 2(2') uncinate, smaller, more erect with shorter base, 100-113 (108, n = 5) long, base 65-70 (68, n = 5) long; hooks 3(3') erect, falcate, 98-110 (105, n = 5) long, base 30-40 (35, n = 5) long; hooks 4(4') to 10(10') slender, erect with recurved tip, short base, gradually diminishing in size along row; hooks 4(4') 108-118 (113, n = 5) long, base 23-35 (30, n = 5) long, hooks 10(10') 80-98 (89, n = 5) long, base 20-43 (30, n = 5) long. Single row of slender 4-7 intercalary hooks, 20-35 (26, n = 5) long, base 5-8 (6, n = 5) long; intercalary hook row begins posterior to hooks 6(6'), terminates between rows 9(9') and 10(10'). External surface of tentacle with chainette of tiny hooks, one chainette element per principal row; chainette hooks at end of principal rows; chainette hooks uncinate, 13-18 (14, n = 5) long. No distinctive basal armature on internal surface;

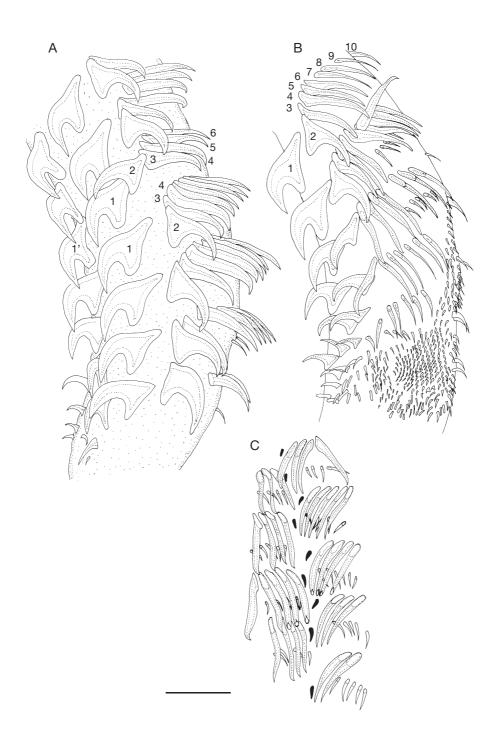


Fig. 3. — Paragrillotia spratti (Campbell & Beveridge, 1993) n. comb. from Negaprion acutidens (Rüppell, 1837): **A**, internal and antibothrial surfaces of tentacle, basal region; **B**, external surface of tentacle, basal region; **C**, mid-region of external surface of tentacle, metabasal region, with chainette elements shown as solid hooks. Scale bar: 10 μm.

disparity between sizes of hooks 1 and 1'; sizes of hooks in ascending series of file 1 from base 70, 73, 100, 108, 115; comparable hooks in file 1' 110, 120, 138, 138, 133 long. On external surface of tentacle, initial rows of small hooks blunt-tipped, 8-18 (14, n = 5) long; anterior to blunt hooks, compact triangular array of small uncinate hooks present; array 12 hooks wide at base, 14 hooks from base to apex; hooks of array 5-10 (8, n = 5) long; from array, band of larger, uncinate hooks extends distally, disappearing in metabasal region; hooks of band 10-15 (12, n = 5) long.

Segments acraspedote, 1.62-3.70 (2.34, n = 10) mm long, 1.10-1.50 (n = 10) mm wide; length: width ratio 1.18:3.08 (1.86, n = 10); genital pores alternate irregularly, lateral, in posterior third of segment margin, 430-1000 (540, n = 10) (27%) from posterior end. Genital atrium with muscular thickenings anteriorly and posteriorly; hermaphroditic sac pyriform, 300-400 (330, n = 10) long, 130-200 (180, n = 10) wide; cirrus sinuous, unarmed, leading to small, often crescentic internal seminal vesicle, 20-70 (35, n = 10) long, 60-120 (90, n = 10); ovoid external seminal vesicle, thickwalled, 45-120 (79, n = 10) long, 40-80 (58, n = 10) wide, leads to coiled vas deferens. Testes numerous (> 600), 38-53 (44, n = 10) in diameter, filling medulla in single layer; confluent anterior to uterus and posterior to ovaries. Vagina joins cirrus in distal region of hermaphroditic sac, runs along posterior border within hermaphroditic sac; at proximal pole of sac, vagina curves posteriorly towards ovarian isthmus; seminal receptacle absent. Ovary 4-lobed in transverse section, lobes 140-240 (180, n = 10) long, 280-410 (340, n = 10) wide;Mehlis' gland posterior to ovarian isthmus, 100-210 (140, n = 10) in diameter. Uterine duct runs anteriorly from Mehlis' to level of hermaphroditic sac; uterus with thick, glandular walls, extends anteriorly to anterior quarter of segment; anterior extremity of uterus extends ventrally to surface of segment forming potential uterine pore; patency of pore not established. Vitelline follicles circummedullary, obscuring internal details of fully mature segments; follicles 20-30 (24, n = 10) in diameter. Ventral osmoregulatory canals 33-50 (42, n = 5) in diameter; narrow transverse canal, 15 in diameter

joins lateral canals at posterior margin of each segment. Dorsal canals not seen in whole mounts or serial sections. Gravid segments absent.

## REMARKS

The present redescription, based on new material from New Caledonia, agrees with the original description of Campbell & Beveridge (1993) in most respects. The scolex is shorter in the new specimens and the tentacle sheaths are coiled rather than sinuous, but this is considered to be due to fixation. In the present collection, two poorly preserved specimens were longer than the others and had the tentacle sheaths extended so that they were sinuous rather than coiled. The measurements of these two specimens (not included in the description) were: scolex 8.1, 8.8 mm long; pars bothrialis 0.92, 1.00 mm long; pars vaginalis 6.2, 6.7 mm long; pars bulbosa 1.50, 1.63 mm long. The lengths of these specimens were comparable with the measurements given in the original description, the main difference being in the longer pars vaginalis. The bulb length was not affected by fixation and the lengths of the bulbs of the newly collected specimens were longer than in the original description (1.50-1.82 mm herein; 1.12-1.28 mm in the original description). A prebulbar organ was not present and the original description is considered to be in error. A ring of muscle around the anterior end of the bulb was apparently mistaken for a prebulbar organ. In the original description, the origin of the retractor muscle could not be determined (Campbell & Beveridge 1993). In the newly collected specimens, the retractor inserts near the anterior extremity of the bulb. The hook sizes in the current redescription are larger than in the original, but this may be due to intraspecific variation. The hooks of paratype specimens were re-examined and the original measurements confirmed. In spite of the differences in measurements, all other features of the tentacular armature were identical with the types.

The principal difference noted in the current description is the presence of a chainette on the external surface of the tentacle. There is one chainette element per principal row of hooks and the chainette elements differ in shape and position from the intercalary row, being aligned with the principal

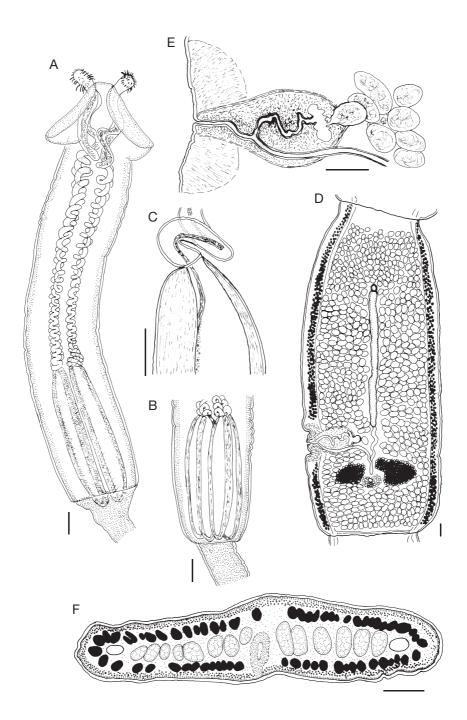


Fig. 4. — Paragrillotia spratti (Campbell & Beveridge, 1993) n. comb. from Negaprion acutidens (Rüppell, 1837):  $\bf A$ , scolex with bulbs projecting into pars proliferans scolecis;  $\bf B$ , posterior region of scolex showing slightly craspedote form, with bulbs not projecting into pars proliferans scolecis;  $\bf C$ , anterior extremity of bulb showing origin of retractor muscle;  $\bf D$ , mature segment;  $\bf E$ , distal genital ducts showing hermaphroditic duct within the hermaphroditic sac;  $\bf F$ , transverse section of mature segment at level of uterine pore. Scale bars: 100  $\mu$ m.

rows rather than posterior to them. On this basis, the species is reallocated to the genus *Paragrillotia*. Some of these elements were illustrated by Campbell & Beveridge (1993: fig. 12) but were presumably not considered to be significant. The presence of the chainette was confirmed by re-examination of paratype specimens.

In the present description, fully mature segments were available and a uterine pore was detected. In addition, a more detailed description of the terminal genital ducts can be given.

Paragrillotia spratti n. comb. differs from both *P. similis* and *P. apecteta* n. sp. in having a single row of intercalary hooks rather than two or three rows, as well as in the basal armature. Paragrillotia apecteta n. sp. has four enlarged hooks in the basal armature which are not present in congeners, while the external surface of the basal armature of *P. spratti* n. comb. bears a compact array of small hooks not present in *P. similis*.

The adult of *P. spratti* n. comb. has previously been reported from the spiral valve of the bronze whaler, *Carcharhinus brachyurus* (Günther, 1870), from the coast of New South Wales, Australia (Campbell & Beveridge 1993) and the plerocercus from the musculature of the teleost *Lisa macrolepis* (Smith, 1846) from the Arabian Gulf by Kardousha (1999).

## DISCUSSION

Dollfus (1969) erected the subgenus *Paragrillotia* for a new species, Grillotia (Paragrillotia) simmondsi Dollfus, 1969 described from Ginglymostoma cirratum from the eastern coast of the United States. The subgenus was distinguished on the basis that the intercalary hook rows were indistinguishable from the band of hooks on the external surface of the tentacle. Schmidt (1986) did not recognise the subgenus, nor did Campbell & Beveridge (1994). Caira & Gavarrino (1990) redescribed the species from specimens collected from the type host and recognised that it was a synonym of Rhynchobothrium simile Linton, 1909. Their redescription of the species, as Grillotia similis, provided detailed information on the anatomy of the segments, hitherto lacking.

Campbell & Beveridge (1993: 41, lines 19-22) in the remarks section describing their new species of *Grillotia*, *G. amblyrhynchos*, indicated that, based on re-examination of the types and the new material of Caira & Gavarrino (1990) of *G. similis*, that a chainette was present on the external surface of the tentacle. Subsequently, Palm (2004) resurrected *Paragrillotia* as a genus, based on the presence of a chainette, with a single species, *P. similis*.

The description of *P. apecteta* n. sp. adds an additional species to the genus, with the new species clearly distinguished by having two or three rows of intercalary hooks and a basal armature differing from that of *P. similis*. Further comparisons are difficult in view of the limited number of specimens of the new species and the fact that fully mature segments were not present.

The redescription of *Pseudogrillotia spratti* based on newly collected specimens indicates that it also possesses a chainette. Although the chainette hooks were illustrated by Campbell & Beveridge (1993), their significance was not realised at the time. The resurrection of the genus *Paragrillotia* with its key distinguishing feature being the presence of a chainette necessitates the transfer of *Pseudogrillotia spratti* to *Paragrillotia*. It is readily distinguished from congeners by the presence of only a single row of intercalary hooks. The details of its segment morphology further confirm the definition of the genus *Paragrillotia* in that, like the type species, it possesses an hermaphroditic duct, internal and external seminal vesicles as well as a uterine pore.

Paragrillotia spratti n. comb. was originally allocated to the genus Pseudogrillotia because the scolex was very slightly craspedote, a key feature then distinguishing Grillotia from Pseudogrillotia. With the abundant new material, it is evident that the scolex can be either slightly craspedote or acraspedote. Similar variation has been noted in the case of Grillotia perelica (Shuler, 1938), currently placed in the genus Pseudogrillotia as it is "slightly craspedote" (Palm 2004). Difficulties in deciding whether a species is "slightly" craspedote or not should mitigate against the use of this character at the generic level.

Palm (2004: 255) subdivided his family Lacistorhynchidae Guiart, 1927 into the Lacistorhynchinae

Guiart, 1927 and the Grillotiinae Dollfus, 1942 based in the presence of a single versus multiple rows of intercalary hooks respectively. *Paragrillotia spratti* n. comb. does not comply with this pattern since it belongs in a genus allocated by Palm (2004) to the Grillotiinae in which there may be either single or multiple rows of hooks.

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